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Prevalence of Donkey Trypanosomosis in Assosa District, Benishangul Gumuz Regional State, Northwest Ethiopia

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Abstract: A cross-sectional study was carried out from November 2014 to April 2015 in Assosa district of Benishangul Regional State, Northwest Ethiopia to estimate the prevalence of donkey trypanosomosis and to identify the species of trypanosome involved. Blood samples were collected from the ear vein of randomly selected 384 donkeys. The buffy coat technique was employed to estimate the prevalence of the disease and pack cell volume (PCV) values was used to assess the disease status (Anemia). Thin blood smear was made from positive samples to identify the species of the parasite. The peasant association (PAs), age, body condition (BCs) and sex of the donkeys were considered to be the risk factors for the occurrence of the disease. Among the examined donkeys, 23 donkeys or 6% (95% of CI: 3.6, 8.4) were infected with trypanosomes. Most of the infections were due to *Trypanosoma congolense* (56.52%) followed by *T. brucei* (30.43%) and *T. vivax* (13%). Univariate logistic regression analysis shows no significant difference in prevalence between PAs, age, body condition and sex. The mean PCV±SD (Standard Deviation) values in parasitaemic animals was 27.4±2.82 and 28.4±2.8 in aparasitaemic animals which is statistically significant ($P<0.05$). In conclusion, the trypanosomosis is an important disease of donkeys. Therefore, further studies should be conducted to determine the impact trypanosomosis poses on donkey and to determine the role of donkeys in the epidemiology of trypanosomosis in general in the area.

Key words: Assosa District · Donkey · Northwest Ethiopia · Prevalence · Trypanosomosis

INTRODUCTION

Ethiopia has an estimated number of 7.03 million equine populations of which 6.75 million are donkeys [1]. Working donkeys help to alleviate poverty by making economic contributions to the household income. This is unhidden fact to Ethiopia as a whole as it encourages national production and improved gross economy of a country. All over Ethiopia, donkeys are primarily used as pack animals. The low level of development of the road transport network and the rough terrain of the country makes donkey the most valuable pack animal under the smallholder farming systems of Ethiopia [2].

Animal trypanosomosis is one of the major impediments to livestock development and agricultural production in Ethiopia contributing negatively to the overall development in general and to food self-reliance efforts of the nation in particular. According to Langridge [3], in Ethiopia the tsetse flies which are the biological vector of trypanosomosis are confined to the southern and western regions between longitudes 33°E and 38°E and latitude 5°N and 12°N. A total of 98,000 km^2 area was infested by five species of tsetse flies until 1976 [3]. In 1980s and 1990s, tsetse flies have progressively out spread from its known belt and invaded the productive agricultural areas in the west and southwest part of the country. Consequently, in the 1990s an estimated area of 180, 000 - 220, 000 km^2 was infested with different species of tsetse flies in which case livestock below 2,000 meter contour are exposed to various levels of trypanosomosis risk [4].

There are many well documented studies addressing the problem of bovine trypanosomosis in Ethiopia but there is currently very little information about equine trypanosomosis regardless of the fact that these animals play a key role in the agricultural economy of the country where poor infrastructure and very ragged topography in many parts of rural Ethiopia have made transportation by vehicle inaccessible.

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The available scant data suggest that trypanosomosis is among the major health constraints of equine in tsetse infested areas of the country. A total of 1.23 million equines are believed to be at risk of contracting trypanosomosis in tsetse infested parts of the country [5]. As Yimam [6] indicated that, the prevalence is 21% in horses in northern Omo Zone, southern Ethiopia. Kanchula and Abebe [7], also reported donkey trypanosomosis with the prevalence of 21% in the same site. Similarly a 28.5% prevalence of donkey trypanosomosis has been reported from Wolayta zone, southern Ethiopia [8, 9]. Recently a prevalence of 6.27% was reported by Mesele and Leta [10] from Dale Wabera District, Western Ethiopia.

On the other hand, all the available reports are from the southern and other part of the country and there are almost no reports from remote States such as BenishangulGumuz Regional State, where there is quite large number of donkey population. The objectives of this study were therefore; (i) to estimate the prevalence of donkey trypanosomosis, (ii) identify the trypanosomes species involved and (iii) identify the risk factor associated.

MATERIALS AND METHODS

Study Area: The study was conducted in randomly selected three PAs (Assosa, Aberahamo and Barro) of Assosa district, Assosa zone, Benishangul Gumuz Regional State. The district is located at a distance of 675kms northwest of Addis Ababa. The study area geographically lies between 8°30' and 40° 27' N latitude and $34^0$21' and $39^0$1' E longitude and 1464 meters altitude above sea level (Figure 1). The climate is characterized by a long rainy season (June-September accounting for 75% of the total rainfall), a short rainy season (February/March to April/May) and a dry season (October-January). The district receives an average rainfall of 950-1000 mm annually and an average annual temperature is around 30°c.

Study Population: The study animals were indigenous breeds of donkeys kept under extensive husbandry which allows free grazing, usually mixed with other livestock in the villages. Donkeys are the main type of pack animals in the area used to transport peoples, water, house hold materials, crops and firewood from one place to another. The equine population in the study area is estimated to be 5, 240what thousands [11].

The age of the selected animals was determined by dentition [12] and body condition scoring was based on neck and shoulders, dorsal and transverse spinous processes of withers, ribs, belly, back, hip bones or hindquarters [13].

Study Design: A cross-sectional type of study was conducted from November 2014 to April 2015 in randomly selected three peasant associations (PAs) of Assosa district, Benshangul Gumuz Regional state to determine the prevalence of donkey trypanosomosis.

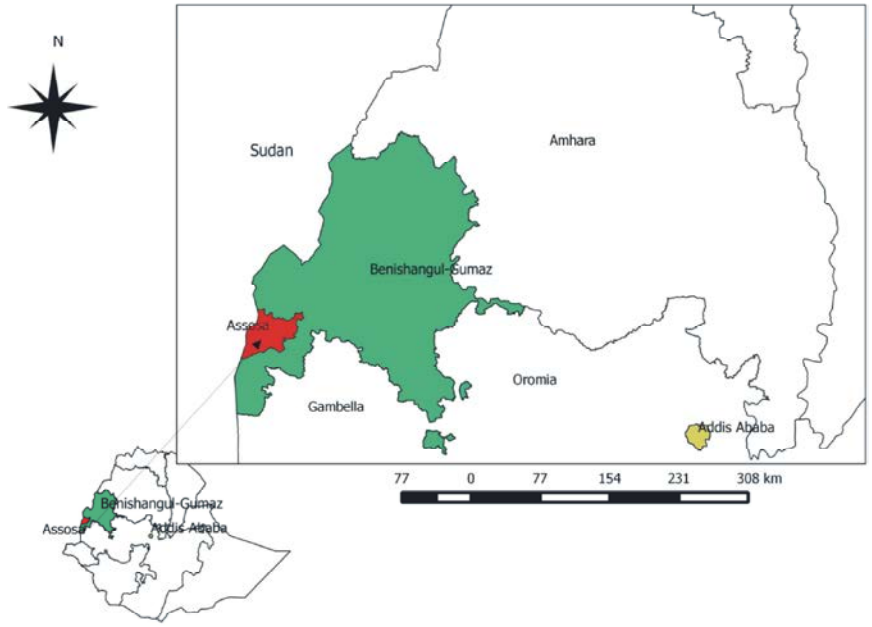

Fig. 1:

Sampling Method and Sample Size Determination: A simple random sampling technique was used to select the study animals. The sample size was determined based on the formula given by Thrusfield n= (1.96^2 x P_{exp}) (1-P_{exp})/d^2). The sample size (n) was on the basis of the expected prevalence (P_{exp}) of 50 % and absolute desired precision (d) of 5% at confidence interval of 95%. As a result, a total of 384 donkeys were sampled [14].

Parasitological and Hematological Examination: Blood samples were collected directly from the ear veins of the study animals using heparinized capillary tubes by puncturing with lancet. The collected blood sample undergo centrifugation, the packed cell volume (PCV) of each sample was estimated using a Hawksley micro-haematocrit reader. After determination of the PCV, the Buffy coat (BC) was examined by dark ground/phase contrast microscope for the detection of trypanosomes in the blood. For the purpose of species identification, a thin blood smear was prepared from the BC for those samples that were positive on BC examination and stained with 10% Giemsa stain and examined under a microscope using the oil immersion 100x objectives following the standard procedure given by Murray *et al.* [15]. Identification of trypanosome species was made by their movement, morphology and their sizes.

Data Analysis: Data collected from each study animals and laboratory analyses were coded and entered into Microsoft excel spread sheet. All statistical analyses were performed using STATA11 software. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individuals' sampled x 100.

The association between prevalence of trypanosome infection and different study variables namely kebele, age, sex and body condition (BCs) was analyzed by univariate logistic regression. Univariate linear regression analysis was used to examine the differences in mean PCV (%) between trypanosome positive and negative animals and to assess the association of different risk factor on the PCV value. In all the analyses, the confidence level was held at 95% and $p<0.05$ was required for significance [14].

RESULTS

Parasitological Finding: The results of buffy coat and Giemsa stained blood smear examination in donkeys are shown in table 1. Out of the 384 donkeys examined, 23 (6%) (95% of CI: 3.6, 8.4) of them were found to be infected with different trypanosome species. Three species of trypanosomes were identified in the study area *T. congolense* 56.5%, *T. brucei* 30.4% and *T. vivax* 13%. Among the study PAs, the highest prevalence was recorded in Baroo (9.3%) followed by Assosa (4.7%) and Aberahamo (4.7%), but the difference was not statistically significant difference ($p>0.05$).

Univariate logistic regression was used to assess whether there is significant difference in prevalence of trypanosomosis in different kebeles, sex and BCs. There is no statistically significant difference ($p>0.05$) in the prevalence of trypanosomosis in different kebeles, sex and BCs (Table 2), Age (Continuous variable) is also found to not affect the prevalence of trypanosomosis significantly ($p>0.05$).

Hematological Finding: The mean PCV±SD value of all donkeys tested was (28.±2.8). Univariate linear regression analysis of PCV with different risk factors is shown in (Table 3). The mean PCV of infected donkeys (27.4±2.8) was significantly lower ($p<0.05$) than that of non-infected donkeys (28.4±2.8) (Table 4).

There was a statistically significant difference in PCV value between male and female donkeys ($P<0.05$). Male donkeys have significantly lower PCV value than female donkeys (Table 3). There was no significant difference in PCV value among animals with different body condition score ($P>0.05$). Age of the animal has also a statistically significant influence on the PCV of the study animals ($P<0.05$). Age and PCV has a positive correlation, as the age increases by one year the PCV value increases by 0.25.

Table 1: The Prevalence of trypanosome infection and species of trypanosomes identified on donkeys in the study Pas.

			Trypanosome spp. %		
Pas	Number of examined	Number of positive	T. brucei	T. congolense	T. vivax
Assosa	127	6	28.6	30.8	-
Baroo	108	10	42.9	53.9	-
Aberahamo	149	7	28.6	15.4	2.1
Total	384	23	30.4	56.5	13

Table 2: Univariate logistic regression analysis of trypanosome infection using different factors.

Factor		Number observation	No. of prevalence%	X2	p-value
PAs	Assosa	127	4.7%	2.64	0.27
	Baroo	108	9.3%		
	Aberahamo	149	4.7%		
Sex	Female	21	14.3%	2.04	0.15
	Male	363	5.5%		
Bcs	Good	6	-	0.75	0.69
	Medium	361	6.1%		
	Poor	17	5.9%		
Age		384	-	0.17	0.68

Table 3: Analysis of the association between mean PCV and the hypothesized risk factors using linear regression.

Factor		Number observation	Mean PCV%	Coef.	p-value
Trypanosome infection	Non-infected	23	28.4	0	0.0122
	Infected	361	27.4	-1.53	
Sex	Female	21	28.9	0	0.0154
	Male	363	28.3	-1.55	
Bcs	Good	6	32.5	0	0.1363
	Medium	361	28.4	-2.076	
	Poor	17	26.4	-1.38	
Age	384	28.3	0.26	0.006	

DISCUSSION

Out of the 384 donkeys examined, 23(6%) of them were found to be infected with different trypanosome species. Three species of trypanosomes were identified in the study district *T. congolense*, *T. brucei* and *T. vivax*. The prevalence in this study was considered to be low when compared with earlier reports from other parts of Ethiopia [7, 16].

This result was in close agreement with the finding of Abebe and Wolde [17], they reported a prevalence of 6.3% from Benishangul Regional State, Northwestern Ethiopia. However, the result of the present study was contrary to the finding of previous reports ranging 18.2-28.5% in different districts of southern Ethiopia [7, 8, 9,16].

The observed differences in prevalence between the present and previous studies can not only be methodological but might be due to reduced tsetse challenge as a result of increased agricultural activities and tsetse control interventions carried out by governmental and non-governmental organizations. Furthermore, investigator competency to detect trypanosomes and variations in geographical and climatic conditions may play a part. Ethiopia is a country where extremes of temperature and rainfall are experienced, altitude being the most important factor [17].

However, the result reported in this study is higher than the result reported by Hailegebrael and Shimelis [18] from Awi zone. They reported a prevalence of 1.6%, Awi zone is found at the edge of tsetse belt area. This difference is might be attributed to this fact as Assosa district is located in the middle of the tsetse belt.

Furthermore, density fly population is another determinant factor for occurrence of trypanosomosis, where fly population increases during long rainy seasons (June to September). However, this study was conducted in November to April which is the dry periods, hence lower fly population and consequently lower prevalence of trypanosomosis expected. In support of this, Sinshaw *et al.* [19] revealed that reproduction and development of biting flies is best suited to the climatic conditions prevalent during the heavy rainy seasons.

In this study, *T. congolense* is found to be the most predominant species followed by *T. brucei* and T. vivax. This result is in agreement with previous reports [8, 16, 20]. This finding also supports earlier observations of Langridge [3] who stated that the savanna tsetse flies (*Glossina morsitans morsitans* and *G. pallidepes*) are more efficient transmitters of *T. congolense* than *T. vivax* in the east Africa. However, it is inconsistent with reports of Kanchula and Abebe [7] and Yimam [6] in which *T. vivax* was reported to be the predominant species. The present finding is also in accordance with reports from Kenya and Gambia [21-24].

Among the study PAs, the highest prevalence was recorded in Baroo (9.3%) than Assosa (4.7%) or Aberahamo (4.7%), but the difference was not statistically significant difference ($p>0.05$). The PAs have similar altitude, soil type, climatic condition, rain fall, vegetation coverage and ambient temperature.

In the present study, the rate of infection was compared among the various categories of age, sex and body condition (BCs). There was no significant difference in the prevalence of trypanosomosis in female animals compared to male animals ($p>0.05$). This result is in agreement with the previously reports [25, 26] and this might be dueto the fact that both sexes have virtually similar exposure to biting flies in grazing areas.

Age is also found to be not significantly associated with trypanosomosis prevalence ($P>0.05$). This is in agreement with the report of Dhollander *et al*. [24]. However it is inconsistent with the work of Abebe and Wolde [17], Ayana *et al*. [25], Solomon *et al*. [27], Tesfaheywet and Abraham [28] and Rowlands *et al.* [29]. As Rowland indicates that the suckling calves are not allowed to go out with their damsuntil they are weaned off [30]. This is not true for foal, as they do not wean through their young life stage with Jennet, so the foal exposed equally for trypanosomosis as adults.

The prevalence of trypanosomosis under different body condition groups is indicated in the Table 2. The infection rate in animals with poor body condition (6.25%) was slightly higher than in animals with good or medium (2.95%) BCs, but it is not significant ($p>0.05$). This study agrees with the work of Abebe and Wolde [17], Solomon *et al.* [27] and Smith [30]. The absence of trypanosome infection in the good body condition animals might be related to the fact that well-nourished animals have good level of immunity and are in a better position to resist infection; moreover there is a very rare possibility of re-establishment of infection in animals with good body condition. Furthermore, as Pinchbeck *et al.* [31] reported that the buffy coat techniques are limited in its capacity of detection in very low parasitemic dose even though the infection is present.

The mean PCV±SD value of all donkeys tested was (28.±2.8). The results of analysis of mean PCV with different factors are shown in Table 4. The mean PCV of infected donkeys (27.4±2.8) was significantly ($p<0.05$) lower than that of non-infected donkeys (28.4±2.8). The decrease in PCV value in parasitemicdonkeys may be due to the simultaneous effect of trypanosomosis, parasitic or other chronic disease, so-it is difficult to associate the lower PCV observed in this study only with trypanosomosis. As Picozzi *et al.* [32] discussed, it might suggest that even though anemia is characteristics of trypanosomosis, other factors such as poor nutrition and the effect of other blood feeding parasites which ultimately cause reduction of PCV. Moreover, the study donkeys were not screened for gastrointestinal, hemoparasites or other chronic disease that cause anemia during the study period. It is, therefore, essential that other anemia producing parasites or other microbial disease are identified and their effects known in order to assess the net effect of trypanosomosis on PCV.

In this study, mean PCV were found to be dependent on sex of the donkeys. Male donkeys have significantly lower ($P<0.05$) PCV value than females. This finding is in agreement with previous reports from Gambia [24] and many other literatures [34, 35]. This variation may be due to the higher magnitude of work load male donkeys possess than female. In the study area farmers often uses male donkeys for carting than female donkeys. However, the result obtained in this study is inconsistent with the work of Abebe and Wolde [17] and Yakubu and Chafe [33].

The body condition score was quite significantly associated with mean PCV ($P<0.05$). It was observed that donkeys in poor body condition had significantly lower mean PCV value than those in good body condition. This study is in agreement with result reported by Abebe and Wolde [17] and Hailegebrael and Shimelis[19]. On the other hand it is inconsistent with the report of Solomon *et al*. [27]. A donkeys having good BCs shows they are in good nutritional management and a relatively high probability to be free from chronic hemoparasite that is why donkeys having good BCs have higher PCV value as compared to poor or medium BCs.

CONCLUSION

Donkey trypanosomosis is an important disease in Assosa district where donkeys are extensively kept. It has negative effect on the PCV of animal, so that trypanosomosis reduce work performance of donkeys. This study revealed that *T. congolense* is the most important trypanosome species as compared to *T. brucei* and *T. vivax* in donkeys in the area. The prevalence observed in this study is generally low however; the study design used should be taken into account as a cross-sectional study depicts only a momentary picture of the infection status. The diagnostic capability of the buffy coat method is also another factor to be considered because the diagnosis of trypanosomosis by direct

parasitological techniques is feasible in the acute state of the illness, when the blood is colonized by a large number of parasites. In the chronic state of the illness which is characterized by low parasitemia, a good parasitological diagnosis is rather difficult. Thus, more sensitive techniques such as serology or PCR should be used for the effective diagnosis of the disease. Therefore, a further longitudinal study that makes use of such techniques and includes entomological survey needs to be conducted in different seasons and agro-ecological zones so as to generate a complete data set on the epidemiology of donkey trypanosomosis in the area.

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